The impact of the Xpert HIV-1 Qual test on early infant diagnosis of HIV in Myanmar and Papua New Guinea: A pragmatic cluster randomised controlled stepped wedge trial

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Summary

Background: Despite proven benefits for child health, coverage of early infant diagnosis of HIV (EID) remains suboptimal in many settings. We aimed to assess the impact of a near point-of-care EID test on time to results communication for HIV-exposed infants.

Methods: We conducted a pragmatic cluster randomised controlled stepped-wedge trial to assess the impact of the Xpert® HIV-1 Qualitative EID test (Cepheid) on time to results communication, compared to standard care laboratory-based testing of dried blood spots using polymerase chain reaction (PCR). Between 1 October 2016 and 31 August 2018, we enrolled HIV-exposed infants at six public health care facilities: four in Myanmar and two in Papua New Guinea (PNG). Health care facilities providing prevention of mother-to-child transmission services were eligible for participation. The primary outcome was to communicate EID results to the infant's caregiver by three months of age. This completed trial was registered with the Australian and New Zealand Clinical Trials Registry, number 12616000734460.

Findings: A total of 393 caregiver-infant pairs were enrolled in the study across both countries. Independent of study time, the Xpert HIV-1 Qualitative test reduced time to EID results communication by 60%, compared to the standard-of-care (adjusted time ratio [ATR] = 0·40, 95%CI=0·29-0·53, p<0·001). In the control phase, 2% (2/102) of study participants received an EID test result by three months of age compared with 74% in the intervention phase (214/291). By six months of age, 58% (59/102) of control participants and 79% of intervention participants had received a test result.

Interpretation: This study reinforces the importance of scaling up near patient care EID testing in resource-constrained and low HIV prevalence settings, typical of the Asia-Pacific region.

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Research in context

Evidence before this study

Early infant diagnosis of HIV (EID) and timely initiation of antiretroviral treatment can significantly reduce mortality and morbidity in children infected with HIV through vertical transmission. However, coverage of EID is inadequate in many settings, particularly in countries with a lower HIV prevalence. We searched PubMed non-systematically using search terms including "point-of-care", "HIV", and "early infant diagnosis" for English language articles published up to December 1st, 2021. No studies from the Asia-Pacific region were found. However, observational studies, randomised controlled trials, and field evaluations from high HIV prevalence African countries (>4·5%) demonstrate the potential of near patient EID testing to improve patient outcomes and reduce HIV-related infant morbidity and mortality in these settings.

Added value of this study

Our study provides evidence that the Xpert HIV-1 Qualitative test for EID is effective in reducing time to results communication in public hospital settings in Papua New Guinea (PNG) and Myanmar, two low HIV prevalence countries in the Asia-Pacific region. It provides novel data to guide the implementation and scale-up of near point-of-care EID in similar contexts.

Implications of all the available evidence

Given the ongoing challenges with equitable access to timely and accurate testing for HIV-exposed infants, the available evidence supports the use of near point-of-care EID testing with nucleic acid tests such as the Xpert HIV-1 Qualitative test. In both high prevalence settings in the African region and low prevalence countries typical of the Asia-Pacific region, point-of-care EID testing can reduce time to results communication to caregivers and clinicians, improve access to timely antiretroviral treatment and potentially reduce infant mortality. Following a global move towards point-of-care platforms, PNG has recently introduced point-of-care EID testing in two high burden provinces with the expectation of further scale-up in the near future. Further research is needed to determine the cost-effectiveness of point-of-care EID testing in low prevalence settings.

Introduction

Despite nearly three decades of HIV prevention efforts, an estimated 1·7 million people are newly infected with HIV each year, including 150,000 children.[1] Delayed HIV diagnoses and sub-optimal coverage of antiretroviral treatment (ART) leading to progression of HIV to AIDS contribute significantly to maternal and infant mortality, particularly in resource-constrained settings.[2] The vast majority of paediatric HIV infections occur through vertical transmission during pregnancy, birth or breastfeeding. Without treatment, rapid onset of AIDS occurs in infants, with up to 50% mortality within two years of infection.[3] Timely ART reduces vertical transmission of HIV to less than 5% even in breastfed children, significantly reducing morbidity and mortality.[3] WHO recommends early infant diagnosis (EID) of HIV within the first two months of life to facilitate treatment initiation in HIV-infected infants.[4]

Virological testing using nucleic acid technologies are recommended for infants younger than 18 months due to the presence of maternal anti-HIV IgG antibodies.[4] While point-of-care nucleic acid testing is recommended, in many settings EID is done using dried blood spots tested at centralised laboratories.[4] Challenges with laboratory testing include the need for specialised equipment, highly trained personnel, and caregivers to return later for EID results.[5] In 2021, 62% of HIV-exposed infants globally received an HIV test within two months of birth, however EID coverage was only 38% in the East Asia and Pacific region.[6] A review of EID in resource-limited settings found significant disparities in the proportion of test results returned to caregivers (ranging from 37-90%) and the time taken to communicate these results (9 days to 21 weeks).[5] Loss to follow-up of infants is a significant challenge, with a systematic review and meta-analysis describing an estimated 25% of HIV-exposed infants in low- and middle-income countries (LMIC) were no longer engaged with EID services at six months of age.[7] A multi-country analysis of UNAIDS data from 56 countries found that countries with HIV prevalence below 4·5% had lower PMTCT coverage, higher vertical transmission, lower EID rates and higher AIDS-related deaths in children compared with higher prevalence countries.[8] While this is likely due to differing health priorities in low prevalence contexts, it highlights the need for more support to HIV programs in these settings.

Point-of-care EID testing offers the potential of same-day results provision, reducing loss to follow-up, and increasing the proportion of HIV-infected infants commenced on treatment in a timely manner. The Xpert® HIV-1 Qualitative assay (Cepheid, Sunnyvale, CA, USA) for EID is widely considered to be a point-of-care or near patient test according to the REASSURED criteria[9], as although it takes 90 minutes, it is able to provide results on the same day as testing.[10] The Xpert test has demonstrated both accurate performance in laboratory and field evaluations and its effectiveness in improving the care of HIV-exposed infants, primarily in high prevalence countries in Africa.[11-13] However, data from low prevalence settings in the Asia-Pacific region are lacking.[14] To address this gap, we conducted a cluster randomised controlled stepped-wedge trial to assess the impact of the Xpert HIV-1 Qualitative EID test on time to results communication among HIV-exposed infants compared with the standard care laboratory test in Myanmar and Papua New Guinea (PNG).

Methods

Study design

This multi-national pragmatic trial used a cluster randomised controlled stepped-wedge design and was conducted at six public hospitals: four in Myanmar and two in PNG. Randomisation was employed at the health facility level due to practical, logistical, and ethical challenges associated with individual-level randomisation. The intervention was introduced in a monotonic time-varying fashion with all facilities starting in a control phase for at least one month, having a one-month training period and then transitioning to an intervention phase. The timing and order of transition into the intervention phase was randomly determined.

Study setting

HIV prevalence in Myanmar is estimated at 0·8% of the adult population, with infections concentrated in key populations and urban areas.[15] The proportion of pregnant women living with HIV who received ART dropped from 85% in 2019 to 18% in 2021, most likely due to political instability, and the vertical transmission rate is currently 30%.[15] Our field trial was implemented in four public hospitals in the Yangon Region (1 October 2016 – 30 June 2018). The hospitals provided prevention of mother-to-child transmission (PMTCT) services integrated into routine antenatal care and were chosen because of their capacity for ART initiation or referral. Two sites (Thingangyun General Hospital; Thanlyin General Hospital) provided ART whereas South Okkalapa Women's and Children's Hospital and Central Women's Hospital referred HIV positive patients to nearby ART centres.

Papua New Guinea has the highest HIV prevalence in Oceania at 0·9% in the adult population, with higher rates among female sex workers and men who have sex with men.[16] An estimated 81% of pregnant women receive ART for PMTCT and 68% of infants receive an HIV test within two months of birth.[16] Final vertical transmission rates of HIV are estimated at 23%.[16] This study was conducted in two public hospitals in PNG from 1 December 2016 to 31 August 2018. Mt. Hagen General Hospital in Western Highlands and Eastern Highlands Provincial Hospital in the Eastern Highlands were chosen due to their relatively high numbers of pregnant women living with HIV attending the PMTCT clinics. Both hospitals provide comprehensive PMTCT services including ART provision for HIV-infected mothers and exposed babies.

Policies in PNG and Myanmar recommend HIV-exposed infants be tested at four to six weeks of age using dried blood spots sent to centralised laboratories. Myanmar has two laboratories with capacity to undertake testing in Yangon and Mandalay, and PNG has one in Port Moresby.

Participants

All infants born to confirmed HIV-infected mothers, aged less than 28 days, and requiring HIV testing were eligible for enrolment. Eligible infants were enrolled if caregivers were aged 18 years or over, were able and willing to provide informed consent for the child to participate and agreed to provide reliable contact information for follow-up purposes.

Ethics approval was obtained from the Alfred Hospital Ethics Committee in Australia; the PNG Institute of Medical Research Institutional Review Board, the National Department of Health Medical Research Advisory Committee, and the Research Advisory Committee of the National AIDS Council Secretariat in PNG; and the Ethical Review Committee of Department of Medical Research in Myanmar. All participants provided written consent in either Myanmar language or Tok Pisin in PNG.

Randomisation and masking

Hospitals were the unit of randomisation for one-way cross-over from control to intervention phases of the study. Each site had between one and ten months of control phase before transitioning (at months 2, 4, 5, 8, 9 and 11) to the intervention, with a total of 33 hospital-months in the control phase and 45 in the intervention phase. The sequencing of facility transition to the intervention was determined at random using computer-based code, with randomisation stratified by country and the facilities de-identified. During the one month of transition and training, no participants were analysed for the study. Given this design and in terms of estimating the effect of the intervention, the intervention was treated as a monotonic time-varying factor in statistical modelling. This trial was non-blinded.

Procedures

Caregivers of eligible infants were invited to participate in the study post-delivery or at the first postnatal visit within 28 days of birth. After enrolment, research staff administered a structured questionnaire on socio-demographic characteristics, obstetric history, health-seeking behaviour, partner's HIV status, and access barriers to care. Two study visits were scheduled to align with the routine care schedule: one at approximately six weeks of age to obtain the infant's blood sample, and one at three to six months of age for provision of HIV test results. All study procedures including blood sample collection took place within the PMTCT clinics. Additional information was collected from caregivers during follow-up visits on experiences with testing procedures, timing and communication of results, infant ART initiation, and infant feeding practices.

In the control phase, enrolled infants had either a heel prick or venous blood sample drawn – depending on local hospital guidelines – for HIV testing at approximately six weeks of age, as per standard care procedures. Up to six dried blood spots were collected for each infant: three to five spots on one card were sent by post to the Central Public Health Laboratory (CPHL) in PNG for HIV PCR testing using the Roche Amplicor HIV-1 DNA Assay (version 1.5; Roche Molecular Systems Inc, Branchburg, NJ, USA), and hand delivered to the National Health Laboratory in Yangon, Myanmar for testing using the Abbott RealTime HIV-1 Qualitative assay (Abbott, Abbott Park, Illinois, USA). The additional blood spot was stored for confirmatory diagnosis if required. The laboratories in both countries had an External Quality Assurance program in place supported by the National Reference Laboratory (Melbourne, Australia; WHO Collaborating Centre for Diagnostics and Laboratory Support for HIV and AIDS).

During the intervention phase, enrolled infants had either a heel prick or venous blood sample taken approximately six weeks after birth for HIV testing using the Xpert HIV-1 Qualitative test. In addition, five dried blood spot specimens (from the same blood draw as for the Xpert test) were collected, four for laboratory-based assay to

assess Xpert test diagnostic performance and confirm diagnosis, and one for storage for quality assurance purposes. All Xpert tests were performed on site at the study hospitals, and wherever possible run on the day the sample was taken. In Myanmar, laboratory staff performed the test and provided results to medical staff who conveyed them to caregivers. In PNG, research nurses hired for this project performed the Xpert test and provided results to caregivers. All test operators attended 2-3 days of comprehensive training on the GeneXpert platform.

As per standard care, hospital staff in Myanmar hand delivered the test results from the central laboratory to clinical teams, and in PNG the laboratory sent results via the postal service – with clinical staff phoning to follow-up on missing results when required. In both study phases, clinicians communicated test results to caregivers at scheduled follow-up appointments as per the standard of care. As per national guidelines, all infants born to HIV-infected mothers were immediately started on prophylactic antiretrovirals. In PNG, infants testing positive were commenced on ART as soon as results were conveyed to caregivers at scheduled follow-up visits. In the two hospitals in Myanmar with ART services, positive infants were commenced on treatment by paediatricians. In the remaining hospitals, infants were referred to external ART clinics. In the intervention phase in Myanmar, treatment was only commenced once a laboratory-based PCR confirmatory test result was obtained. All infants testing negative were scheduled for further testing at nine and 18 months using standard care laboratory testing, and after cessation of breastfeeding.

For ethical reasons, enrolled infants with caregivers who did not attend scheduled visits for sample collection after three months of age were actively followed up by the study team, and similarly for those caregivers who did not come for results communication after six months of age. In both the control and intervention phases, study staff attempted to contact participants twice by phone and then by visiting their place of residence once (with consent) to remind them to attend the clinic.

Trained research staff collected all quantitative data on paper forms including questionnaires and HIV test result forms. Double data entry was undertaken using REDCap electronic data capture tools hosted at Burnet Institute in Australia.

Qualitative data collection was undertaken with health workers, caregivers, and key informants within the PMTCT program. Cost-effectiveness data were collected in PNG.

Outcomes

The primary outcome was HIV-exposed infants and their caregivers' receipt of an infant HIV test result by three months of age.

The secondary outcomes were:

1. Caregivers' receipt of an infant HIV test result by six months of age
2. Field performance of the Xpert HIV-1 Qualitative test compared with laboratory-based PCR
3. Operational feasibility and acceptability of the Xpert test (reported elsewhere)[6,17,18]
4. Cost-effectiveness of the Xpert test compared with standard of care in PNG (results still to be published)

Statistical analysis

Based on available records, an estimated five infants would be enrolled each month per site. Over a 14-month study duration, we estimated that approximately 390 HIV exposed infants (260 participants from four facilities in Myanmar, and 130 from two facilities in PNG) would be enrolled. Power was estimated separately for Myanmar and PNG. For Myanmar, based on the assumption that approximately 50% of infants receive an EID test result by three months of age, we estimated the study was powered (80%, 5% significance, intraclass correlation coefficient [ICC]=0·05) to detect an increased three-month EID test result receipt of 84% due to the intervention (relative rate ratio=3·6). For PNG, assuming the same three-month test receipt proportion, ICC, Type I and II error rates, the study was powered to detect an increased three-month EID test result receipt of 94% (relative rate ratio=12·2). Power was based on the estimation of an intervention effect from a stepped-wedge cluster randomised design assuming analysis by generalized linear mixed modelling (GLMM)[19], and incorporated the specific study design characteristics (training periods, block sizes and number of block transitions).

Given the dependencies in the data due to the stepped-wedge study design (also known as clustering), a crossed random effects GLMM was applied using generalised structural equation modelling (GSEM). GSEM permits the estimation of a broad range of generalised linear models and complex multilevel mixed effects variance structures where dependencies are present in the data. The study design was such that two levels were present in the data; at

level-2 random effects were specified for both facility and time (month) and these were crossed at the infant observation level (level-1). Given the inherent time component of the primary outcome, we undertook parametric survival analysis to estimate the effect of the Xpert HIV-1 Qualitative test and provide inference using an accelerated failure time (AFT) model, assuming a log-normal distribution for the baseline hazard for time to receipt of the test. These primary outcome analyses were 'intention-to-treat' in nature with a participant's intervention status in statistical modelling based on their facility's randomised stepped-wedge intervention phase, regardless of which EID test they actually received. Akaike's information criterion (AIC) and Bayesian information criterion (BIC) were used to compare various distributional assumptions (log-normal, Weibull, Gamma, log-logistic) for the baseline hazard in terms of model fit. In terms of the fixed component of the survival model, we estimated monotonic time-varying terms for the intervention and time (quadratic) – the latter term a necessary model condition to ensure estimates of the intervention effect (given its monotonic nature) are not confounded with any observed changes in general test result expediency during the study. In the context of the survival modelling, the data in this study were right-censored (infants who had not received a test by 3-months of age – including those who did not return for sampling or communication of test results) and left-truncated (infants came under observation [enrolled into the study] with different post-birth durations). The maximum likelihood estimation method used in the survival modelling produces unbiased effect estimates in the presence of right-censored data. To provide unbiased estimation in the presence of left-truncated infant test receipt events (also termed 'delayed-entry'), survival modelling was such that infants contributed to the likelihood (i.e. were included in the risk set) from their date-of-enrolment, not date-of-birth. To determine the extent to which country-specific effects of the introduction of the Xpert test were present in the trial (i.e. that the intervention functioned differently between Myanmar and PNG), we also estimated an intervention by country interaction term to provide country-specific estimates of the intervention. Survival probabilities using the fixed components from the GLMMs (i.e. where the survival probability estimates set the random effect means for facility and month to zero – therefore representing survival probabilities for an *average facility* and *average month* in terms of time to EID test receipt) were produced and plotted to compare the time to EID test result receipt between control and intervention study phases.

We also undertook parametric survival analysis applying an 'as-treated' type approach using the methods described above. In these analyses, the intervention status of the participant in statistical modelling was based on the actual test modality they received, not the intervention phase as part of the randomised stepped-wedge design. To this end, in the 'as-treated' statistical analysis the treatment of the intervention departs from the monotonic time-varying nature based on the facility randomised stepped-wedge design specified in the trial protocol and simply varies based on the test an individual participant received at any given time during the study.

To assess test performance of the Xpert HIV-1 Qualitative test, correlation of values compared to gold standard PCR reference tests was assessed through the determination of misclassification, sensitivity, specificity, and negative and positive predictive value, and reported following Standards for Reporting of Diagnostic Accuracy (STARD) guidelines. The failure rate of the Xpert test was determined as the proportion of performed tests during which no test result could be established either through an indeterminate result or test failure (invalid test). It was not possible to obtain the proportion of invalid PCR test results from the centralised laboratories. We used Stata version 13.0 for all data analysis.

This study was registered with the Australian and New Zealand Clinical Trials Registry, number 12616000734460.

Role of the funding source

The funder of the study had no role in study design, data collection, analysis, interpretation, or manuscript writing. Cepheid (Sunnyvale, CA) donated GeneXpert machines and test cartridges to the project but had no role in study design, data collection, analysis, interpretation, or manuscript writing

Results

Figure 1 provides a flow diagram according to the CONSORT statement: 2012 extension to cluster randomised trials.[20] A total of 416 participants were enrolled across six health facilities – 226 participants in two sites in PNG and 190 participants from four sites in Myanmar. Three participants withdrew from the study and 20 received an EID test during the one-month training and transition phase and were not included in the analysis. Another 42 infants were enrolled during the control phase but returned to the study facilities for EID testing during the intervention phase. These infants erroneously received standard care testing (28 in Myanmar and 14 in PNG) but

were necessarily treated as intervention phase participants in the intention-to-treat analysis. These participants were treated as control participants in the as-treated analysis.

Key characteristics of the 393 caregiver-infant pairs enrolled across the six clinics are shown in Table 1 – 102 in the control phase and 291 in the intervention phase. A higher proportion of study participants tested in the control phase were from Myanmar (62% [63/102]) compared with PNG (38% [39/102]), and the median age of infants at enrolment was higher in the control phase (5 days versus 3 days). All other demographic characteristics were similar between control and intervention participants.

Sixteen percent of study participants (62/393) were not retained during follow-up (control phase 12% [12/102]; intervention phase 17% [50/291]). This included 42 participants (11%) who did not come back for a blood draw after enrolment (control phase: 0% [0/102]; intervention phase: 14% [42/291]) and 20 (6%) who did not return for a test result (control phase: 12% [12/102]; intervention phase: 3% [8/291]).

In the control phase, 2% (2/102) of study participants received a test result by three months of age compared with 74% (214/291) in the intervention phase. By six months of age, 58% (59/102) of control participants and 79% (231/291) of intervention participants had received a test result.

We observed that the Xpert HIV-1 Qualitative test reduced time to receipt of an EID test result by 60%, compared to the standard-of-care, and this difference was statistically significant (adjusted time ratio [ATR]=0·40, 95%CI=0·29-0·53, $p<0·001$; Table 2). Across both study phases, there was a general non-linear reduction in time to receipt of an EID test result as the study progressed such that the effect of study time in reducing test receipt duration was strongest earlier in the trial (linear term: ATR=0·83, 95%CI=0·72, 0·95), square term: ATR=1·01, 95%CI=1·00-1·02; Wald $\chi^2(2)=8·54$, $p=0·014$; Table 2). A similar reduction in time to receipt of an EID test result in the intervention phase was observed at 6-months (ATR=0·44, 95%CI=0·34-0·57, $p<0·001$; Table 3) and although there was a general reduction in time to receipt of an EID test result across both study phases, as there was for the 3-month censored analyses (Table 3), the quadratic term for time was not statistically significant (Wald $\chi^2(2)=1.64$, $p=0·44$) . The as-treated analysis also showed a statistically significant reduction in time to receipt of an EID test result in the intervention phase at 3-months (ATR=0.33, 95%CI=0.28-0.40, $p<0·001$) and 6-months (ATR=0.21, 95%CI=0.17-0.26, $p<0·001$).

In additional GSEM survival analyses, the intervention effect was permitted to vary across country through estimation of an intervention by country interaction. The intervention exhibited greater impact in reducing test receipt duration in PNG (Myanmar; ATR=0·54, 95%CI=0·40-0·72; PNG: ATR=0·31, 95%CI=0·22-0·43), however the difference was not statistically significant (Wald $\chi^2(1)=0·88$, $p=0·35$).

A higher proportion of infants in the control phase received a blood draw for EID testing by three months of age (97% [99/102] versus 80% in the intervention phase [233/291]) (Table 4). Of these participants, more infants in the intervention phase received an EID result (control: 79% [78/99]; intervention: 97% [225/233]). In Myanmar, 73% of control participants (45/62) received a test result by six months of age versus 100% of intervention participants (82/82). In PNG, 38% of infants in the control group (14/37) received an EID result by six months of age versus 93% in the intervention group (141/151). Among participants who received a blood draw by three months of age, 19% of those in the control group received an EID test result after six months of age (19/99) versus 2% (1/233) in the intervention phase; 21% of control participants received no EID test result (21/99) versus 3% in the intervention phase (8/233).

Diagnostic accuracy of the Xpert test is presented as a flow diagram according to STARD guidelines (Figure 3).[21] Of the 223 Xpert tests performed, 200 were HIV not detected (90%), eight were HIV detected (4%), and 15 were invalid (7%). Repeat Xpert testing on all invalid tests using the same blood sample detected no HIV infection. Of the intervention participants who received an Xpert test, 28% (62/223) did not receive a standard care PCR result within six months of study completion; all these participants were in PNG. Reference standard testing using PCR was performed on 163 of the 223 samples with an Xpert test result: 161 infants were HIV not detected and two HIV detected. All PCR results were concordant with the Xpert test. The diagnostic performance of the Xpert HIV-1 Qualitative test showed 100% sensitivity (95%CI=16-100) and 100% specificity (95%CI=98-100). Most Xpert tests (93%; 207/223) were performed on the day of blood sample collection; remaining tests were performed the following day. Seven of the eight infants found to be HIV infected were immediately commenced on ART or referred for treatment, in line with national guidelines. The remaining infant died before the caregiver received their test result. The caregiver was not able to wait at the clinic for a same-day result. Study staff contacted the caregiver by phone, but they did not return to the clinic for the result until after the infant had died.

In this pragmatic trial, no safety and adverse events were reported related to the diagnostic testing intervention. Any positive and negative attributes of the test are reported separately.[6,17,18]

Discussion

Our study found that implementation of the Xpert HIV-1 Qualitative test for EID in public hospitals in PNG and Myanmar resulted in a statistically significant reduction in time to results communication to caregivers of exposed infants. This is the first study in a low HIV prevalence LMIC setting in the Asia-Pacific region showing the impact of the Xpert test for EID. Same-day EID testing has consistently been shown to improve access to timely HIV test results. A study in eight African countries found that point-of-care EID reduced median times from sample collection to return of results from 55 to 0 days and increased the proportion of infants receiving results within 30 days (18·7% versus 98·3%).[11] Our study showed that Xpert testing led to more infants receiving HIV test results within three and six months of age. The potential impact of point-of-care EID testing was clearest in PNG, where significant delays in communication of PCR results back to study sites meant that 62% of control participants did not receive EID results by six months of age.

Providing timely EID results to caregivers of HIV-exposed infants has important and proven benefits for child health. Early diagnosis of HIV in children facilitates timely treatment initiation, reducing morbidity and mortality.[3] During study implementation, two of the sites in Yangon did not have the facilities to provide paediatric ART to infants found to be HIV-infected, preventing staff from commencing same-day treatment even in the absence of a need for confirmatory testing. These two hospitals have since set up paediatric ART clinics in their facilities, maximising the potential impact of the Xpert HIV-1 Qualitative test on same-day treatment initiation for HIV-infected infants. Studies in Africa have consistently demonstrated that the use of point-of-care EID leads to earlier commencement of ART and it is likely that this would also be the case in Myanmar.[11,22,23] In study sites in PNG, clinic nurses could prescribe paediatric ART and were able to commence treatment immediately for six out of the seven infants with a positive Xpert test.

Reducing the time between testing and results communication will potentially have broader benefits for families beyond early treatment initiation. Quicker turnaround of results could provide welcome reassurance to caregivers of both HIV-infected and HIV-uninfected infants.[5] Conversely, while all mothers in this study had been diagnosed with HIV before their most recent pregnancy, it is conceivable that for women dealing with a new diagnosis, early provision of positive HIV results for their infant could be challenging.[24] Our project did explore the acceptability of the Xpert test, including provision of same-day results. Both caregivers and health care providers found the test be acceptable and no concerns were identified about the provision of same-day results. More detailed qualitative findings are presented elsewhere.[6,17,18]

Very few participants were lost to follow-up after blood collection, with 93% of infants in Myanmar and 90% of infants in PNG receiving an EID result. This is promising for improving the health of children born to women living with HIV. Other studies show a large disparity in the proportion of caregivers receiving EID test results for their children, ranging from 37% in Tanzania to 81% in Botswana.[5,25-28] These differences could be caused by a range of factors including a difference in the overall HIV prevalence in the country leading to differing investment in HIV services[8], as well as differences in quality and effectiveness of laboratory and health information systems.[5]

While the presence of a point-of-care EID test is essential for providing same-day results, the availability of the GeneXpert platform in hospital settings is not always sufficient. Not all study participants who underwent an Xpert test were provided with results on the same day as testing, including one infant found to be HIV-infected. Reasons included caregivers not being able to wait for results, clinic staff not having time to run the tests if participants presented late in the afternoon and challenges integrating the Xpert HIV-1 test into routine clinical care.[17,18] Similar challenges have been documented with same-day results provision for other point-of-care tests.[13,25] Qualitative findings from this project suggest that strengthening infrastructure and streamlining communication pathways would improve the feasibility of same-day results provision by providing staff with a dedicated space to run tests and provide results, and by scheduling patients early in the day.[17,18]

The field performance of the Xpert test was comparable to laboratory PCR testing in both countries, with a sensitivity and specificity of 100%. When interpreting our findings on diagnostic accuracy, it should be noted that we identified very few positive cases, and 26% (58/223) of the standard care laboratory test results were not available during the data collection period. Despite these limitations, studies in other settings have also demonstrated the high accuracy of the Xpert HIV-1 Qualitative test, with 100% sensitivity and 99·9% specificity in a field study in South Africa[13], 94·1% sensitivity and 99·8% specificity in Kenya[12], and 100% agreement between the Xpert HIV-1 test and PCR in Malawi.[29] Invalid Xpert test results occurred in 7% of runs,

predominantly due to inaccuracies with the test procedure or inconsistent electricity supply at study sites. Other field evaluations of the Xpert HIV-1 Qualitative test show similar error rates with 8·7% of samples not providing an interpretable result in a study in Malawi[29] and 5% of tests resulting in errors in South Africa.[13]

This study had several limitations that impact interpretation of the findings. Firstly, due to logistics and feasibility of implementation, 42 study participants who enrolled during the control phase but came for testing during the intervention phase did not receive the Xpert HIV-1 Qualitative test for EID but instead received only the standard of care. In addition to our primary outcome intention-to-treat analysis we also estimated the effect of the intervention using an as-treated type analysis – where participants were analysed according to the actual test they received, regardless of the randomised facility intervention status. Our as-treated analysis also showed a statistically significant reduction in time to results communication at both 3 and 6 months of age, strengthening the validity of our study findings.

Secondly, in PNG, research staff were hired specifically for study implementation, including running the Xpert test. This makes it difficult to assess feasibility of integrating the test into routine clinical care using existing resources. However, no additional staff were hired for the study in Myanmar, suggesting feasibility in this context. Thirdly, this study was undertaken in two provinces in PNG and one region of Myanmar. Given the ethnic and cultural diversity in both countries, the results of the study may not be generalisable, particularly to areas with greater health system constraints.

Compared with the standard care laboratory PCR test, the Xpert HIV-1 Qualitative test for EID performed well and resulted in a reduction in time to communication of results to caregivers in both Myanmar and PNG. Given the disparity of access to HIV services in many lower prevalence settings, point-of-care EID testing holds considerable promise for reducing infant morbidity and mortality in both Myanmar and PNG, and potentially in other LMIC across the Asia-Pacific region. More information is needed on the health system implementation requirements of the Xpert test for EID in lower-level health facilities, as well as the cost-effectiveness of implementing point-of-care EID in low prevalence settings.

Data sharing

Individual participant data that underlie the results reported in this article (text, tables, and figures), after de-identification, and the study protocol are available up to seven years following publication of this article to researchers who provide a methodologically sound proposal. Proposals should be directed to the Alfred Hospital HREC (research@alfred.org.au; Project 500/14).

Contributors

SL, SC, DA, CN, XSC and MS conceptualised the study design. AV, AKH, HH and YM oversaw study implementation; WLY, JG, AK, SS and GM were responsible for data collection. SB provided technical support and training. PA led the statistical analysis; SL, YM, WLY and MDP contributed to data cleaning and analysis. YM led the manuscript writing. SL, WLY, YM, AV, AKH, SB and JG contributed to data interpretation. SL, YM and PA directly accessed and verified the underlying data reported in the manuscript; SL had final responsibility for the decision to submit for publication. All authors (YM, HH, AV, AKH, WLY, JG, PA, SB, MDP, CN, XSC, ZK, AK, GM, SS, WT, TMZ, LLK, ZK, MS, SC, DA, HHT and SL) read and approved the final manuscript.

Declarations of interest

We declare no competing interests.

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References

1. UNAIDS. Global HIV & AIDS statistics — 2020 fact sheet. 2020. https://www.unaids.org/en/resources/fact-sheet#:~:text=In%202019%2C%20there%20were%2038.0,(0%E2%80%9314%20years). (accessed 11 Dec 2021).
2. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* 2016; **388**(10063): 3027-35.
3. Violari A, Cotton MF, Gibb DM, et al. Early antiretroviral therapy and mortality among HIV-infected infants. *N Engl J Med* 2008; **359**(21): 2233-44.
4. World Health Organization. Updated recommendations on HIV prevention, infant diagnosis, antiretroviral initiation and monitoring: March 2021. 2021. https://www.who.int/publications/i/item/9789240022232 (accessed 9 Oct 2022).
5. Ciaranello AL, Park JE, Ramirez-Avila L, Freedberg KA, Walensky RP, Leroy V. Early infant HIV-1 diagnosis programs in resource-limited settings: opportunities for improved outcomes and more cost-effective interventions. *BMC Med* 2011; **9**: 59.
6. Yee WL, Than KK, Mohamed Y, et al. Caregiver experience and perceived acceptability of a novel near point-of-care early infant HIV diagnostic test among caregivers enrolled in the PMTCT program, Myanmar: A qualitative study. *Plos One* 2020; **15**(10).
7. Carlucci JG, Liu Y, Friedman H, et al. Attrition of HIV-exposed infants from early infant diagnosis services in low- and middle-income countries: a systematic review and meta-analysis. *J Int AIDS Soc* 2018; **21**(11): e25209.
8. Kempton J, Hill A, Levi JA, Heath K, Pozniak A. Most new HIV infections, vertical transmissions and AIDS-related deaths occur in lower-prevalence countries. *Journal of Virus Eradication* 2019; **5**: 92-101.
9. Land KJ, Boeras DI, Chen XS, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nat Microbiol* 2019; **4**(1): 46-54.
10. World Health Organization. Novel Point-of-care tools for Early Infant Diagnosis of HIV. 2017. http://www.who.int/hiv/pub/toolkits/early-infant-diagnosis-hiv-2017/en/ (accessed 18 Dec 2021).
11. Bianchi F, Cohn J, Sacks E, et al. Evaluation of a routine point-of-care intervention for early infant diagnosis of HIV: an observational study in eight African countries. *Lancet HIV* 2019; **6**(6): e373-e81.
12. Opollo VS, Nikuze A, Ben-Farhat J, et al. Field evaluation of near point of care Cepheid GeneXpert HIV-1 Qual for early infant diagnosis. *PLoS One* 2018; **13**(12): e0209778.
13. Technau K-G, Kuhn L, Coovadia A, Murnane PM, Sherman G. Xpert HIV-1 point-of-care test for neonatal diagnosis of HIV in the birth testing programme of a maternity hospital: a field evaluation study. *The Lancet HIV* 2017; **4**(10): e442-e8.
14. Diallo K, Modi S, Hurlston M, Beard RS, Nkengasong JN. A Proposed Framework for the Implementation of Early Infant Diagnosis Point-of-Care. *AIDS Res Hum Retroviruses* 2017; **33**(3): 203-10.
15. UNAIDS. Country factsheets: Myanmar 2021. 2021. https://www.unaids.org/en/regionscountries/countries/myanmar (accessed 9 Oct 2022).
16. UNAIDS. Country factsheets: Papua New Guinea 2019. 2019. https://www.unaids.org/en/regionscountries/countries/papuanewguinea (accessed 15 Nov 2021).
17. Mohamed Y, Kupul M, Gare J, et al. Feasibility and acceptability of implementing early infant diagnosis of HIV in Papua New Guinea at the point of care: a qualitative exploration of health worker and key informant perspectives. *BMJ Open* 2020; **10**(11).
18. Yee WL, Htay H, Mohamed Y, et al. Operational experiences associated with the implementation of near point-of-care early infant diagnosis of HIV in Myanmar: a qualitative study. *BMC Health Serv Res* 2021; **21**(1): 863.
19. Hemming K, Lilford R, Girling AJ. Stepped-wedge cluster randomised controlled trials: a generic framework including parallel and multiple-level designs. *Stat Med* 2015; **34**(2): 181-96.
20. Campbell MK, Piaggio G, Elbourne DR, Altman DG, Group C. Consort 2010 statement: extension to cluster randomised trials. *BMJ* 2012; **345**: e5661.
21. Cohen JF, Korevaar DA, Altman DG, et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. *BMJ Open* 2016; **6**(11): e012799-e.

22. Leach-Lemens C. Evaluating point-of-care early infant diagnosis in Zambia and Zimbabwe. 2020. https://www.aidsmap.com/news/mar-2020/evaluating-point-care-early-infant-diagnosis-zambia-and-zimbabwe (accessed 16 October 2020).
23. Mwenda R, Fong Y, Magombo T, et al. Significant Patient Impact Observed Upon Implementation of Point-Of-Care Early Infant Diagnosis Technologies in an Observational Study in Malawi. *Clin Infect Dis* 2018.
24. Varga CA, Sherman GG, Maphosa J, Jones SA. Psychosocial consequences of early diagnosis of HIV status in vertically exposed infants in Johannesburg, South Africa. *Health Care Women Int* 2005; **26**(5): 387-97.
25. Dube Q, Dow A, Chirambo C, et al. Implementing early infant diagnosis of HIV infection at the primary care level: experiences and challenges in Malawi. *Bull World Health Organ* 2012; **90**(9): 699-704.
26. Mugambi ML, Deo S, Kekitiinwa A, Kiyaga C, Singer ME. Do diagnosis delays impact receipt of test results? Evidence from the HIV early infant diagnosis program in Uganda. *PloS one* 2013; **8**(11): e78891.
27. Nuwagaba-Biribonwoha H, Werq-Semo B, Abdallah A, et al. Introducing a multi-site program for early diagnosis of HIV infection among HIV-exposed infants in Tanzania. *BMC pediatrics* 2010; **10**: 44.
28. Sundaram M, Lukhele B. Identification patient loss points from testing to treatment initiation among infants tested in Swaziland. 5th IAS Conference on HIV Pathogenesis and Treatment. Cape Town; 2009.
29. Ceffa S, Luhanga R, Andreotti M, et al. Comparison of the Cepheid GeneXpert and Abbott M2000 HIV-1 real time molecular assays for monitoring HIV-1 viral load and detecting HIV-1 infection. *J Virol Methods* 2016; **229**: 35-9.

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